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## **Tracking the fate of individual cells following exposure to heat stress**

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The most fundamental question to ask about any organism is whether it is alive or dead, but providing an answer to this question is far from easy. The term “viable” is generally used to describe the ability of a cell to undergo proliferation (Postgate, 1969) and is typically determined via plate counts. The plate count method is routine in microbiology laboratories world-wide and while this may work to an acceptable extent in laboratory situations, it is far from suitable for the analysis of environmental samples (Rappe and Giovannoni, 2003). Other, so-called rapid methods, use stains to attempt to equate an instantly measurable parameter (stain uptake, exclusion or processing) to its viability. Stains may indicate membrane permeability, enzyme activity etc that are characteristics of a cell that might be expected to be able to form a colony under the right conditions but the relationship is far from absolute. The attempt taken, either visually using a microscope or electronically by flow cytometry, is to set a threshold that discriminates live from dead. However, should we make this binary distinction? In many cases the “health” or “vitality” of a cell may be important and the stages that a cell may pass through as it passes from a live, fully functional, replicating cell to a dead cell are various and depend on the reason for death. Starvation, presence of toxic chemicals and unsuitable temperatures will all be characterised by different impacts whether we are considering bacterial cells or multicellular eukaryotes. Consequently one measure of “death” and a clean line between live and dead is unlikely to be appropriate, especially in environmental samples where multiple stressors and heterogeneity of response are commonplace.

Attempts to categorise cells as alive, dead and in-between (Davey, 2011) are problematic but important. A damaged but recoverable cell may be sufficient to re-establish a population after a pollution incident or extreme weather event. A damaged and dying cell may still be able to cause

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damage to a host through release of endotoxins. Consequently, understanding more about the route from life to death at the single cell level and the extent to which this can be reversed through resuscitation or recovery will inform developments in antimicrobial therapy, bioremediation and microbial biodiversity in a changing environment.

As reported in this issue (Govers, *et al.*), intracellular movement of protein aggregates can be used to provide an indication of the metabolic status of individual cells of *Escherichia coli*. Movement of the protein aggregates was measured in individual cells using repeated imaging techniques during the application of heat stress. Many studies have investigated the impact of heat stress on microorganisms but a common limitation is that measurements are typically taken at fixed time points before and after the stress is applied. Furthermore, experimentation is often at the level of the population rather than the individual, yet we know that stress and response to it is very heterogeneous even in nominally clonal populations. Govers *et al.* were able to determine not only whether a particular cell survived but also to track cell fates through alive -> damaged -> recovered or alive -> damaged -> dead. By following individual cells in this way the existence of a “late-resuscitating” phenotype was identified. This leaves an unanswered question about whether such cells are extensively damaged but recoverable or whether they are employing a bet-hedging strategy that may result from more careful and complete damage repair. Either way, this is a big step forward from assays that measure only at fixed time points before and after the application of stress.

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Govers *et al* This issue

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